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Studies on *Neostromboceros albicomus* (Konow) (Hymenoptera: Tenthredinidae), a Potential Biological Control Agent for the Old World Climbing Fern, with Notes on Two Other Species of *Neostromboceros*

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Abstract.—Three species of *Neostromboceros* have been reared from ferns in Thailand, Vietnam, Malaysia, and Indonesia. The biology, taxonomy, and distribution are presented for *N. albicomus* (Konow) on *Lygodium* spp. (Lygodiaceae), *N. congener* (Konow) on *Christella arida* (Thelypteridaceae), and *N. luchti* Malaise on *Diplazium asperum* (Athyriaceae). *Neostromboceros albicomus* is a potential biological control agent for the Old World climbing fern, *Lygodium microphyllum* (Cavanilles) R. Brown, an invasive plant in southeastern United States. In the field, *N. albicomus* occurs on both *L. flexuosum* and *L. microphyllum* but attempts to rear insects from one plant host on the other were not successful. DNA sequencing of *N. albicomus* from the two hosts showed a single base difference between the two groups, indicating that two biotypes of *N. albicomus* may exist. ***Stromboceros* (*Neostromboceros*) *metallica* Rohwer 1912** is a **new synonym** of ***Neostromboceros albicomus* (Konow 1901).**

Lygodium microphyllum (Cavanilles) R. Brown (Lygodiaceae), the Old World climbing fern (also known as the small-leafed climbing fern) native to southeastern Asia, is an invasive weed in the Everglades of Florida and is a target species for a USDA/ARS biological control program. During a search for biological control agents of this fern in southeastern Asia, three species of the genus *Neostromboceros* Rohwer were discovered feeding on ferns of the genera *Lygodium*, *Diplazium* (Athyriaceae), and *Christella* (Thelypteridaceae). Because so little is known of these sawfly species, and because of the potential for biological control by one of them, we present some data on their taxonomy, distribution, hosts, and life history.

The genus *Neostromboceros* is represented by about 45 species and occurs from Japan south to Papua New Guinea and Indonesia west to China, Nepal, and India (Malaise 1944, Naito 1979, Smith unpublished). It is one of the largest genera of the subfamily Selandriinae in this region, but nothing was known of its hosts and habits except for three of the six species in Japan, one of which feeds on *Athyrium japonicus* Copel, and two of which feed on *Athyrium* sp. (Athyriaceae) (Naito 1979). Since most Selandriinae feed on ferns and some adults of *Neostromboceros* have been collected from ferns, it has been assumed the larval host plants of most or all species are ferns. Malaise (1944) stated that adults are always found on or near lower ferns

in moist places, and inferred that ferns should be the food plant of the larvae.

Molecular characterization is increasingly being used as a method of indicating species diversity, identifying cryptic species, and matching immature stages with adults (Pemberton and Ferriter 1998, Goolsby et al. 2000). In this study, most collections of *Neostromboceros albicomus* (Konow), the most promising species for biological control and found throughout Thailand, Malaysia, and Vietnam, were the larval stage. Because larvae cannot be characterized morphologically, DNA sequencing was used to determine species status. This is discussed in the methods section and was used to determine larval identity and the distribution of *N. albicomus*.

METHODS

For identification of *Neostromboceros albicomus*, we sequenced the D2 expansion domain of the 28S rRNA which has proved useful for all life stages of insects and mites. Other genes such as ITS may be sequenced if finer resolution below the species level is needed, but this is much slower and more expensive than the automated sequencing of D2. The methods are those described by De Barro et al. (2000).

The polymerase chain reaction (PCR) was used to amplify the D2 gene regions for each specimen. Primers for the region followed Campbell et al. (1993); D2F 5′-CG TGTTGCTTGATAGTGCAGC-3′ and D2R 5′-TTGGTCCGTGTTTCAAGACGG-3′, or ND2F 5′-AGTACCGTGAGGGAAAGTTG-3′, which was used in some reactions as an alternate forward primer which anneals approximately 90 bases down stream of the D2F binding site. All reaction volumes were 50μL, containing 20pM of each primer, 200mM each dGTP, dATP, dCTP, and dTTP, 1.5–2.5mM MgC12, 2μL DNA lysate, 1X supplied buffer and 2.5U Taq polymerase (Bresatec, Australia). PCR amplification was done using a Hybaid thermocycler using the following parameters. A pre-cycle denaturation step for 5 min at 94°C, followed by the addition of the Taq polymerase. Then, 35 cycles of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C followed by a final post-cycle extension step at 72°C.

Molecular characterization was used to determine species status for *Neostromboceros albicomus* larvae from *Lygodium*. *Neostromboceros congener* (Forsius) and *N. luchti* Malaise were not studied further because they were not found on the target food plant; however, we record new food plant data, biology, and distribution that we have available. The information given for *N. luchti* is an independent study by RDdeC.

Acronyms used are as follows: DEI = Deutsches Entomologisches Institut, Eberswalde, Germany; USNM = National Museum of Natural History, Smithsonian Institution, Washington, DC., USA.

SPECIES

Neostromboceros albicomus (Konow)

(Figs. 1–6)

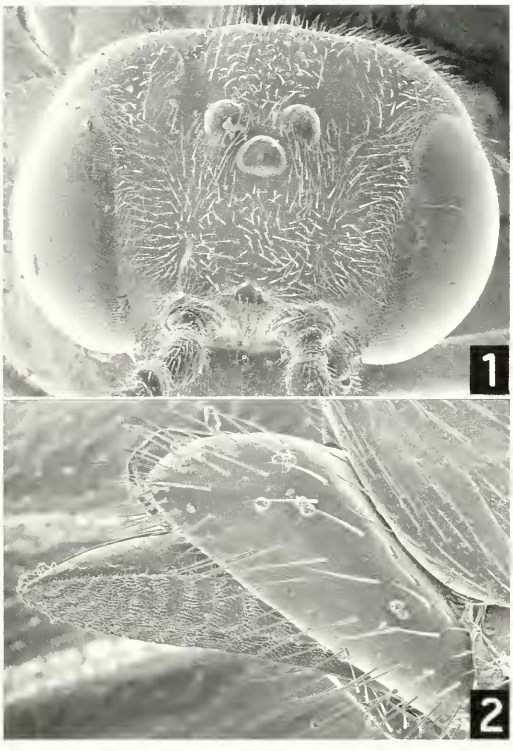
Stromboceros albicomus Konow 1901: 65.

Neostromboceros albicomus: Forsius 1933: 169, 183 (Malaysian records); Malaise 1944: 45 (syn.: *S. cenchralis* Konow).

Stromboceros cenchralis Konow 1908: 149.

Stromboceros (*Neostromboceros*) *metallica* Rohwer 1912: 236; Forsius 1933: 169; Malaise 1944: 44. **New synonymy.**

Recognition.—Adults (Fig. 3) black, abdomen with middle tergites reddish, sometimes appearing as central band; wings hyaline with apical part of forewing beyond stigma infuscate. Third antennal segment longer than 4th; antenna round, not compressed, slightly incrassinate in middle. Anterior margin of clypeus slightly emarginate; head smooth and shiny, without punctures (Fig. 1); without antennal furrows lateral to frontal area; antennal sockets not carinate; lateral supra-antennal pits circular, connected by a short furrow to antennal sockets; malar space



Figs. 1–2. *Neostromboceros albicomus*. 1, Head, frontodorsal view. 2, Sawsheath and ovipositor.

linear; postocellar area broader than long; head strongly narrowing behind eyes. Mesopleuron smooth, shining; epicnemium indistinct, almost wanting. Female sheath slender, from above of uniform width; sheath and ovipositor as in Fig. 2.

This is one of the few species of *Neostromboceros* with part of the abdomen red; most species have a black abdomen with the posterior margin of the segments narrowly white. The above characters will separate this species from other species with part of the abdomen red.

Discussion.—Forsius (1933) mentioned that *N. albicomus* and *N. metallicus* were probably synonymous. Malaise (1944) separated *N. metallicus* and *N. albicomus* (= *cenchralis*) in his key to species, both going to the same couplet. He did not see Rohwer's type of *metallicus* (mentioning "after Rohwer"), so he was unable to compare it with the type of *N. albicomus*. He mentioned seeing both types (*albicomus* and *cenchralis*), and two females. In a footnote, he stated "That *N. metallicus* is really specifically different from *albicomus* is uncertain and needs confirmation." DRS compared types of *metallicus* and *albicomus* side-by-side and concluded that *N. metallicus* is a new synonym of *N. albicomus*; the previous synonymy of *chechralis* is also confirmed.

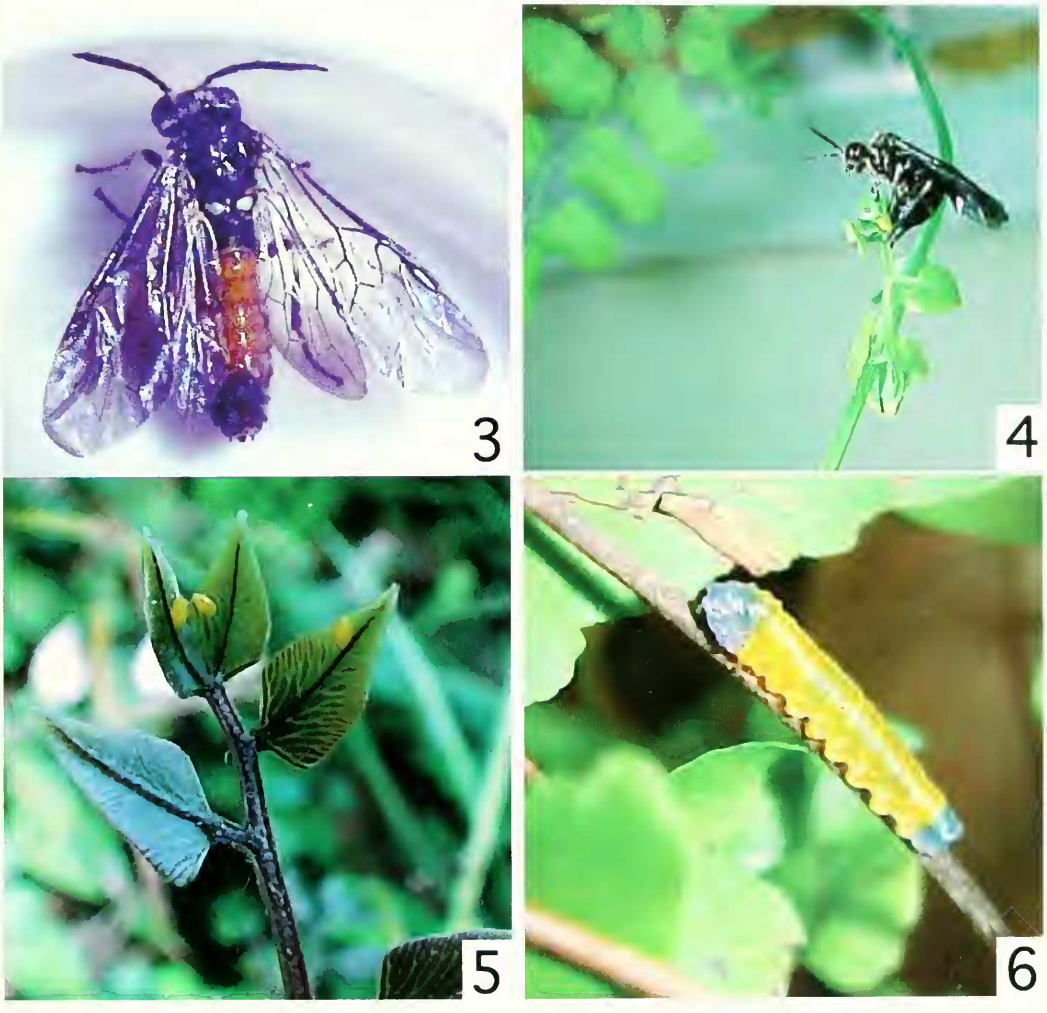
Konow (1901) described *Stromboceros albicomus* from "Malacca (Perak)" and stated it was in the "Mus. Hung." He described the female but did not give the number of specimens he had. We examined one syntype in the DEI labeled "Perak," "Coll. Konow," "Syntypus," "Stromboceros albicomus Knw., Perak."

Konow (1908) described *Stromboceros cenchralis* from "Insulae Philippinae (Palawan)." He described the female but did not state how many specimens he had. We examined one female in the DEI labeled "Palawan," "Coll. Konow," "Holotypus," "Stromboceros cenchralis Knw., Ins. Philipp."

Rohwer (1912) described *Stromboceros* (*Neostromboceros*) *metallica* from "Singapore, Malay Peninsula. One female collected February 25, 1909, by Bryant and Palmer." It is the type species of *Neostromboceros* Rohwer, described as a new subgenus. The holotype in the USNM is labeled: "Singapore Malay Penin," "Bryant & Palmer Coll.," "Hym Slide 307," "wing mounted," "♀ Type No. 14505 U.S.N.M.," "Neostromboceros metallicus Roh., TYPE ♀."

Hosts.—*Lygodium flexuosum* (Linnaeus) Swartz and *L. microphyllum* (Lygodiaceae). Larvae fed and completed their live cycle on *L. salicifolium* Presl, but adults did not oviposit on this species.

Biology.—AW maintained colonies of this "*Lygodium* sawfly." Larvae were collected on *Lygodium flexuosum* growing along roadsides at Hui Nam Rin, Wiang Pa Pao District, Chiang Rai Province in northern Thailand and taken to the labo-



Figs. 3–6. *Neostromboceros albicornus*. 3, Female. 4, Female ovipositing on *Lygodium flexuosum*. 5, Eggs on *L. flexuosum* shoots. 6, Larva on *L. flexuosum*. Photos by A. D. Wright and A. Winotai.

ratory in Bangkok. During preliminary biological studies at 25°C, unmated females laid eggs (Fig. 4) a few hours after emergence. Yellow oval eggs were laid singly on young shoots and leaves (Fig. 5) and became orange yellow before hatching. Eye spots were visible through the chorion. Newly hatched larvae were pale with dark head capsules. Large larvae were yellow with small purple bands at the anterior and posterior ends of their bodies (Fig. 6). Full grown larvae moved into the soil where they formed cocoons and pupated. In laboratory studies, adult sawflies were fed a honey solution. Duration studies indicated a preoviposition period of 1–2 hours and an egg incubation period of 3–5 days. Some larvae had 4 instars and some 5 instars, but it was not determined if the numbers related to sexes. The total larval period was 20–22 days and the pupal period was 15–21 days. The adult longevity was 3–4 days.

Observations on feeding and oviposition behavior of *N. albicornus* indicated differences according to whether larvae were collected on *Lygodium flexuosum* or on *L. microphyllum*. The presence on two food

plants suggest that *N. albicomus* may have two biotypes so far indistinguishable by morphological taxonomy, and this appears to be supported by results of DNA sequence results referred to in the distribution section below. Sequencing was done on eight specimens collected from *L. flexuosum* and three from *L. microphyllum*. The sequences separated into two groups with a single base difference between them, according to the host plant. Within each group, there was no variation in the sequenced D2 gene. Sequences for the two groups are deposited in GenBank, accession numbers AF453417 and AF 453418.

(A) Collections on *L. flexuosum*: Observations on feeding behavior indicated that *N. albicomus* larvae collected on *L. flexuosum* preferred feeding only on *L. flexuosum*. Limited host-specificity testing indicated the sawfly fed and completed its life cycle on *L. microphyllum* and *L. salicifolium*, but it did not feed on ten ornamental ferns of the genera *Adiantum* (Adiantaceae), *Asplenium* (Aspleniaceae), *Nepholepis* (Nephrolepidaceae), *Davallia* (Davalliaceae), or *Pteris* (Pteridaceae). In both Bangkok and Brisbane studies, adults appeared reluctant to oviposit on *L. microphyllum*, and, though larvae placed on *L. microphyllum* survived, they took longer to develop, there was high mortality, and emerged adults did not lay any eggs. Although *L. japonicum* (Thunberg) Swartz has yet to be tested with larvae collected on *L. flexuosum*, we speculate it may also be a suitable host plant of *N. albicomus*, since the morphological similarity of *L. flexuosum*, *L. salicifolium*, and *L. japonicum* indicates they are probably closely related (P. Bostock, pers. comm.).

(B) Collections on *L. microphyllum*: Observations indicated *N. albicomus* collected on *L. microphyllum* preferred feeding only on *L. microphyllum*. When supplied *L. japonicum*, both cut foliage and whole plants, larvae failed to feed and died. Adults provided with a choice of *L. microphyllum*, *L. japonicum*, and *L. flexuosum* laid most eggs on *L. microphyllum*, none on *L. japonicum*, and only three were seen on *L. flexuosum*.

Distribution.—The following records are confirmed by us. DNA sequencing was carried out on specimens (mostly larvae) collected at various places from Thailand, Malaysia, and Vietnam, and results indicated all were the same species, with exact sequence matches (collections from *Lygodium flexuosum*) denoted by a single asterisk (*) and at the same single base difference (in collections from *Lygodium microphyllum*) denoted by a double asterisk (**). MALAYSIA: Selangor: Kuala Lumpur, 1 August 1983, G.F. Hevel & W. E. Steiner (1); Perak, Kuala Woh, 1 September 1992, leg. D.G. Furth (1); *Kuala Lumpur, Taman Cheras Muda, 3° 06.2′N, 101° 05.2′E, 4 July 1999, larvae on *Lygodium flexuosum*, H. L. Ho; Kuala Lumpur, Cheras, Taman Seraya, 3° 06.9′N, 101°45.4′E, 8 November 1999, larvae on *Lygodium flexuosum*, A. D. Wright & H. L. Ho; *Kedah, Langkawi, 5 June 1999, larvae on *Lygodium flexuosum*, H. L. Ho; *Pahang, 22 km E of Maran on highway #2, 3° 40.7′N, 102° 54.9′E, 12 August 1999, larvae on *Lygodium fleuxosum*, A. D. Wright & H. L. Ho. PHILIPINES: Palawan. SINGAPORE: ''Singapore.'' THAILAND: *Chiang Rai Province, Wiang Pa Po District, nr. Huai Nam Rin, side road ca. 70 km NE of Chiang Mai on road #1019, 19° 05.9′N, 99°27.5′E, 30 August 1998, larvae on *Lygodium flexuosum*, A. D. Wright & A.Winotai; *Surat Thani Province, Don Sak District, roadwide nr. Ban Pang Nga Shee, 9° 13.5′N, 99°39.3′E, 1 December 1998, eggs and larvae on *Lygodium flexuosum* (nearby *L. microphyllum* had no sawflies or sawfly damage), A. Winotai & A. D. Wright (reared 1 ♀); *Narathiwat Province, Yi-ngo District, rubber plantation in Luhbohlausa, 6° 25.5′N, 101° 41.7′E, 20 April 1999, 8 August 1999, larvae on *Lygodium flexuosum*; Narathiwat Province, Tak Bai District, 25 February 2001, larva on *Lygodium microphyllum*, A. Winotai & A. D. Wright;

*Chiang Mai Province, San Sai District, nr. Ban Pong, 18° 55.3'N, 99° 2.9'E, 27 April 1999, 29 April 1999, 5 August 1999, eggs and young larvae on *Lygodium flexuosum* (4 larvae with tachinid eggs, 4 tachnid adults emerged in quarantine, Brisbane), A. Winotai & A. D. Wright; *Rayong Province, Kao Chamao Subdistrict, nr. Klong Pla Kang Waterfall, nr. Samkor Village, 12° 56.0'N, 101° 42.9'E, 13 May 1999, larvae on *Lygodium flexuosum*, A. Winotai & A. D. Wright; Trat Province, Klong Yai District, Tambol Mai Root, Ban Huang Som, 11°50.1'N, 102° 50.6'E, 3 April 2001, larvae on *Lygodium microphyllum*, A. Winotai; **Trat Province, Klong Yai District, Tambol Mai Root, Ban Huang Som, 11° 50.1'N, 102° 50.6', 3 April 2001, larvae on *Lygodium microphyllum*, A. D. Wright & A. Winotai. VIETNAM: **nr. Ho Chi Minh City, larvae on *Lygodium microphyllum*, 23 October 1996, Thai Van.

Malaise (1944) gave the distribution as: "The Malay Peninsula (Perak; Keday [Gurum; Catchment Area near Jitra]; West Coast [Langkawi Island; Pulo Pinang]; East Coast [Pulo Aor]); The Philippines (Palawan)." Forsius (1933) recorded: Malay Peninsula: Kedah, Garun, November–December 1916; Keday, Catchment Area near Jitra, 7–10 April 1928; Malay Peninsula, West Coast, Langkawa Islands, 19 April–1 May 1928, 20 August 1928.

Neostromboceros congener (Konow)

Stromboceros congener Konow 1901: 64.

Neostromboceros congener: Forsius 1934: 110 (Java); Malaise 1944: 40 (syn.: *S. karnyi* Forsius).

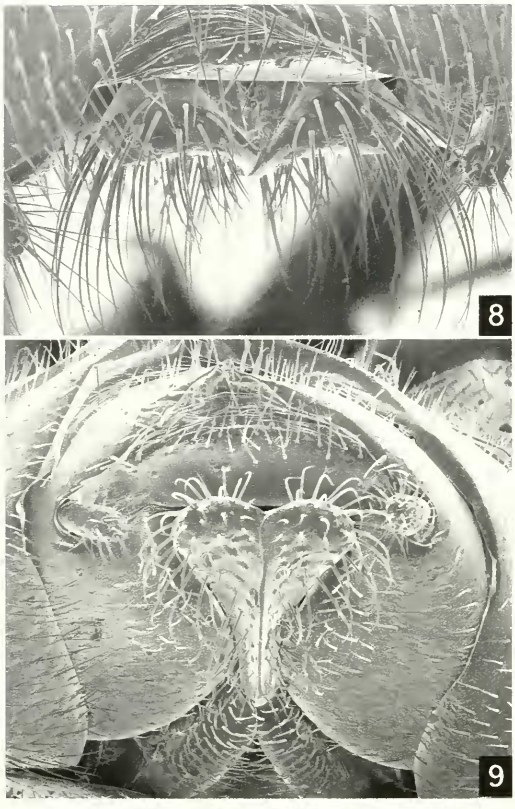
Stromboceros karnyi Forsius 1931: 33; Forsius 1934: 107, 110 (additional distribution records).

Recognition.—Adults black with following white: labrum, posterior margin of pronotum, anterior margin of tegula, perapterum, narrow posterior margin of tergites, apices of coxae, trochanters, apices of femora, and basal part of tibiae; wings hyaline, forewing only slightly subinfuscate toward apex. Antenna almost round in cross section, not distinctly compressed but faintly more compressed in male; flagellar joints without hair-brushes; 3rd segment slightly longer than 4th. Clypeus truncate or with very shallow anterior emargination; malar space linear; head smooth and shining, without punctures; no distinct furrows lateral to frontal area; lateral ocellar furrows distinct; postocellar area subquadrate, slightly broader than long; inner margins of eyes parallel in female, slightly converging below in male. Thorax and abdomen smooth and shiny, without punctures. Female sawsheath slender, in dorsal view of uniform width.

Fig. 7. *Neostromboceros luchti*, frontodorsal view of head.

The black coloration with the above parts white, lack of punctures on the head and body, nearly truncate clypeus, and the third antennal segment slightly longer than the fourth will recognize this species. The above characters will separate this species from other species that are mostly black and lack punctures on the frontal area.

Discussion.—Konow (1901) described *Stromboceros congener* from "Lombok (Sapit)" and stated it was in "Mus. Hung." He did not state how many specimens he had, but he described both sexes. One female at DEI is labeled "Lombok, Sapit 2000', Mai-Juni 1896, H. Fruhstorfer," "Coll. Konow," "Syntypus," "Stromboceros congener Knw., Lombok." Also one female



Figs. 8–9. *Neostromboceros luchti*. 8, Sawsheath, dorsal view. 9, Sawsheath, posterior view.

bears the same data but lacks a determination label. Two other females are labeled "Kelantin," "Coll. Konow," but they cannot be types because the locality differs from that of the original description.

Malaise (1944), in his key to *Neostromboceros*, mentioned seeing 30 females and 15 males of *congener* (= *karnyi* Forsius), some of them compared with the types.

Host.—*Christella arida* (D. Don) Holttum (Thelypteridaceae), collected by ADW in northern Sumatra (identity confirmed by P. Bostok).

Distribution.—We examined specimens from the following: INDIA: Buxar Duar. Bengal, D. Nowrojee, 5.1907 (1); Kobo, 400 ft., Arbor Exped., 3-XII-11, Kemp (1); Sadiya, 21–25 May 1920, Fletcher Coll. (2); "India" 1952, G. W. Angelet (1). INDONESIA: Lombok, Sapit, 2000'; North Sumatra, roadside on track near Lake Toba, 2° 44.7'N, 98° 53.2'E, 22 May 1999, A. D. Wright, collector, adults resting and larvae feeding on ferns, 2 adults and 1 larva on fern *Christella arida*, A. D. Wright & R. Desmier de Chenon. MALAYSIA: Up.-Perak, 1902 (1).

Malaise (1944) recorded the distribution as "Lombok; Eastern Java (Bondowoso, 1–1500 m.; G. Raoeng, 450–700 m.; etc.)." Forsius (1934) gave "Zwei Weibschen un drei Männchen aus Buitenzorg, Dezember 1931. Ein Männchen aus Malang, November 1931." Forsius (1931) described *S. karnyi* from "Java, Tjibodas, 1400 bis 1500 m."

Neostromboceros luchti Malaise
(Figs. 7–14)

Neostromboceros luchti Malaise 1944: 31.

Recognition.—Adults (Fig. 10) black with purplish tinge and with following white: labrum, posterior margin of pronotum, perapterum, trochanters, and basal stripe on tibiae; wings hyaline with apical half of forewing infuscate from base of stigma. Head with frontal area almost flat, distinctly punctured or rugose with punctures confined to frontal area (Fig. 7). Clypeus truncate; malar space linear; postocellar area broader than long, subconvex; lateral postocellar furrows convexly curved, reaching back of head; middle supra-antennal pit transversely furrow-like, almost straight and shallow; lateral furrows rounded or semicircular with tubercle in middle; lower half of hind orbits carinated. Mesopleuron smooth, shiny, im-

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Figs. 10–14. *Neostromboceros luchti*. 10, Female. 11, Eggs on *Diplazium asperum*. 12, Larva. 13, Larvae feeding on *D. asperum*. 14, Cocoon. Photos by R. Desmier de Chenon.


10


11


12


13


14

punctate. Female sawsheath strongly dilated at apex (Figs. 8, 9).

The black coloration with the above parts white, punctured frontal area of the head, and strongly dilated female sawsheath will distinguish this species. The above characters are intended to separated this species from other species that are mostly black and have punctures on the frontal area.

Host.—*Diplazium asperum* Blume (Athyriaceae) (identity confirmed by P. Bostok).

Biology.—This species was reared in Indonesia by RDdeC. The host is a common understory fern in oil palm plantations. The complete cycle from egg to adult takes about 22 to 30 days to complete. Incubation period for the eggs is three to four days, larval development about 20 days, and the time from pupation to eclosion is about 9 to 10 days. The male goes through five instars, and the female six instars. Results of feeding experiments indicate that a single larva consumes about 29 square centimeters of plant tissue during its development. The average number of eggs per dissected female is about 59 with a range of 40 to 98 in 14 specimens dissected. The size of the eggs ranges from 1.12 to 1.18 mm in length and 0.36 to 0.46 mm in width. Eggs are laid on the underside of the frond (Fig. 11), and the number of eggs laid per frond averages about 64, about 41% of which hatched. The larva (Fig. 12) is shiny, green with a darker green dorsum, and many can be found feeding on a fern frond (Fig. 13). The cocoon in the ground is made up of particles of soil (Fig. 14).

Distribution.—INDONESIA: Sumatra, Province of North Sumatra, Bagun Bandar Estate (oil palm plantation), 3°19.62'N, 99°01.76'E (rearings by RDdeC); "Java (Bondowoso, 1000–1500 m; G. Raoeng, 450–700 m, Buitenzort)" (Malaise 1944). MALAYSIA: Kuala Lumpur (Malaise 1944).

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